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CHEMICAL COMPOSITION, ANTIMICROBIAL AND INSECTICIDAL ACTIVITIES OF *CITRUS PARADISI* PEEL ESSENTIAL OIL FROM ALGERIA

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ABSTRACT

The essential oil obtained by microwave-assisted hydro-distillation (MAHD) and hydrodistillation (HD) techniques from the peel of grapefruit (*Citrus paradisi*. L) from Algeria was analyzed by gas chromatography–flame ionization detector (GC–FID) and gas chromatography ⁄ mass spectrometry (GC⁄ MS). Twenty-one constituents were identified in essential oils obtained by HD and twenty-eight constituents in essential oils by MAHD, representing respectively 99.11% and 99.74% of the total oil. The main constituents were limonene (85.54%-87.51%) for MAHD and HD, β-myrcene (2.99%-3.24%), nootkatone (1.78%-1.80%). The antimicrobial activity of the essential oils was evaluated by disc diffusion method. The results showed interested inhibition growth against the tested bacteria (*Escherichia coli*, *Pseudomonas aeruginos, and Staphylococcus aureus*)and the yeast (*Candida albicans),*with an inhibition zone ranging from 4 to 20 mm.Both essentials oils showed no effect against *Aspergilusniger*, *Verticillium sp* and *Thielaviopsis sp.* The insecticidal activity was investigated against adults *ceratitis capitata*. Both essential oils showed a toxic effect against this insect (LD_{50}:9.12µl EO/ml of acetone; LD_{90}: 13.18 µl EO/ml of acetone).

Keywords: *Citrus paradisi*, essential oil, hydrodistillation, microwave-assisted hydro-distillation, antimicrobial activity, insecticidal activity, *Ciratitis Capitata*

INTRODUCTION

Citrus paradisi (Rutaceae) has greater value in human diet **(Malik, 1994)**, popularly called grapefruit, its tree grows up to 3-5 m hig and its fruit is mostly big and globular in bright yellow or lemon colored. The grapefruit is believed to have arisen from the pomelo or shaddock (*Citrus grandis*) or as a hybrid between pomelo and sweet orange **(Bender and Bender, 2009; Adenge, 2007)**. *Citrus paradisi* is a rich source of bioactive molecules including flavonoids, sugars, organic acids, phenolic compounds, steroids and triterpenoids **(Kelebek, 2010; Zhang *et al*., 2011).** The peel oil has a strong and desirable aroma widely used in industrial flavoring of foods, beverages, pharmaceutical products, perfumes and cosmetics, as is common with other citrus oil **(Pisano, 1986)**.
Many researches have been carried out on the chemical composition of grapefruits essential oils obtained by hydrodistilation **(Caccioni et *al*., 1998; Ferhat *et al*., 2016)**, microwave **(Uysal *et al*., 2011; Ferhat *et al*., 2016)**, cold press **(Ferhat *et al*., 2016; Kirbaslar *et al*., 2009)** or micro-wave hydrodiffusion **(Jorge and Sánchez, 2000)** belonging to different region in the world. These studies revealed the limonene as major compound in both oils. Whereas other components such α-pinene, β-pinene, sabinene, myrcene, γ-terpinene, nootkatoone, octanal, nonanal, decanal, geraniol and geranial were present on remarkable amounts. It is interesting to note that the essential oils investigated have various compositions due to the origin, development stage of the collected plant material, seasonal, environmental and experimental conditions.
Grapefruits extract and their compounds are known to exhibit various biological activities including antioxidant **(Bousbia *et al*., 2009; Ghasemi *et al*., 2009; Jun Yu *et al*., 2005)**, antibacterial **(Karioti *et al*., 2012; Jun Yu *et al*., 2005; Benavente-Garcia *et al*., 1997)**, anticancer **(Kotamballi *et al*., 2012)**, anti-inflammatory, antiallergenic, analgesic **(Viuda-Martos et *al*., 2007; Tsujiyama *et al*., 2013)**, antifungal (**Karioti *et al*., 2012; Viuda-Martos *et al*., 2008)** and insecticidal **(Giatropoulos *et al*., 2012)**. In addition the citrus peel extract demonstrate the inhibitory effect of the Adipogenesis caused from high fat-induced dio model **(Karagozlu *et al*., 2016)**.

However we have not found any information on the biological effects of essential oil from *Citrus paradisi* peels of Algerian origin. The aim of this study was the investigation of the chemical composition of this essential oil obtained by hydro-distillation assisted by microwave heating and hydrodistillation, respectively. Therefore, the comparison of the two techniques in terms of yields and composition were reported. The antimicrobial activity of the essential oils was tested against three bacteria (*Escherichia coli*, *Pseudomonas aeruginosand Staphylococcus aureus*), the yeast *Candida albicans* and three fungi (*Verticillium sp., Verticilliumsp*and *Thielaviopsis*). The insecticidal activity of the peel of *Citrus paradisi*essential oil obtained by hydrodistillation was investigated against adults *ceratitis capitata.*To the best of our knowledge, this investigation can be considered as the first information on the study of insecticidal activity of grapefruit peel essential oil from Algeria.

MATERIAL AND METHODS

Plant material

In this study, 5 Kg of fruits of *Citrus paradisi* were collected on February 2015 from the horticultural station located at Mitidja area, 50 km west of Algiers (Bougara, Algeria) (36°31'59.99" N and 3°04'60.00" E). After collection, the peel was separated from the freshly picked fruits and chopped into 1cm pieces, before used for extraction.

Essential oil

MAHD process

The peel of the fresh grapefruit was removed and 100 g was weighed and added into 1 L round bottom flask containing 50 mL of water. The oil extraction was carried out at atmospheric pressure for 30 min. The flask was connected to a Clevenger-type apparatus located outside the microwave oven. In this process, we had to add some distilled water in the extraction flask. The Essential oil was

removed from the aqueous medium by a simple decantation. After separation, the essential oil was dried over anhydrous sodium sulphate and stored at 4 °C until used for analysis. MAHD was performed three times and the mean value was reported.

HD process

The peel of the fresh grapefruit was removed and 100 g was weighed and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus; with 500 ml of water. The essential oil was removed from the aqueous medium by a simple decantation. After separation, the essential oil was dried over anhydrous sodium sulphate and stored at 4°C until used for analysis. HD was performed three times and the mean value was reported.

Gas chromatography and gas chromatography coupled to mass spectrometry analyses

GC–FID system (Agilent, CA, USA, 2000) was used for GC analysis fitted with a fused-silica capillary column containing a non-polar stationary phase HP-5ms (30 m × 0.25 mm × 0.25 µm film thickness). The column temperature program was 60°C for 8 min, then was increased at 2°C/min to 250°C and held at 250 °C for 15 min. A 0.2 µL of the essential oil was injected into the splitless GC inlet. Injector and detector temperature were kept at 250°C. The carrier gas was nitrogen flowing through the column at 0.3 µL/min.
GC–MS (Agilent Technologies) system comprising a 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness), coupled to a quadrupole HP mass spectrometer (Agilent Technologies) MSD 5973, was operated using the following conditions: carrier gas, He; flow rate, 0.3 mL/min, splitless mode; injected volume, 0.2 µL of essential oil; injection temperature, 250 °C. The used ionization mode was electronic impact at 70 eV. The ion source and interface temperature were 230 and 250 °C, respectively, mass range was 30-600 m/z. Oven temperature program was the same given above for GC. The homologous n-alkane series C_5–C_{28}injected in GC–MS under the same conditions as the essential oils were used to calculate the retention indices.
The constituents of the oil were identified by using standard reference compounds and also by matching the mass spectra fragmentation pattern with National Institute of Standards and Technology (NIST) Mass Spectra Library stored in the GC-MS database.
The retention indices of the volatile extract constituents compared with those of the published index data **(ADAMS, 2007)** are used to confirm the identification.

Antimicrobial activity

The antimicrobial activity of the essential oils was evaluated by disc diffusion method as described by Bauer **(Bauer *et al*., 1969)** using Mueller-Hinton agar for bacteria and SAB (Sabouraud) agar for fungi and yeast with determination of inhibition zones.

Microbial strains

The antimicrobial activity of the essential oils was tested against a panel of microorganisms and fungi including *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, the Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 (SARM), three filamentous fungi, *Aspergillusniger*ATCC 16404, *Verticilliumdahliae*,*Thielaviopsis*spp., and the yeast *Candida albicans* ATCC 10231.

Disc diffusion method

Bacterial and yeast suspensions were prepared from colonies resuspended in 10 mL of NaCl (0.9%), and homogenized to obtain an inoculum with an opacity of 0.5 F (~ 10^8 cells/ mL). After spreading these suspensions by sterile swabs on Mueller Hinton agar and Sabouraud agar respectively, sterile standard empty antibiotic discs (6 mm in diameter) were impregnated with various volumes (10, 30 and 50 µL) of each essential oil and then deposited on the surface of Petri dishes. The incubation was carried out at 37°C overnight. The antibacterial and anti-yeast activities were estimated by measuring the diameter of growth inhibition around the disks, DMSO and Erythromycin were used as negative and positive control; each assay was performed three times.
To evaluate the antifungal activity, 2 µL containing approximately 10^4 spores x mL^{-1} were spotted onto Sabouraud agar at 1.5 cm distance from the impregnated discs. The dishes were incubated at 28 °C for 3 days. The antifungal activities were monitored by the ratio of the fungal growth diameter in absence and presence of the essential oil. Each assay was performed three times.

Insecticidal activity

Insect culture

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the mostnotorious insect pests of citrus species causing extensive fruit losses worldwide **(Mavrikakis *et al*., 2000).** This insect is a highly polyphagous species, having more than 300 host fruits **(Liquido *et al*., 1990).**
Strain of *Ceratitis capitata* (*C. capitata*) used in this study was obtained from the laboratory of I.N.R.A.A (National Institute of Agronomic Research of Algeria).
Adults of *C. capitata* were kept in parallelepiped cage (40x50x40cm), covered by a fine mesh cloth for ventilation, with easy access to liquid solution containing a mixture of water, sugar, citric and benzoic acid on a cotton wick.
The culture was placed at 26 ± 2 °C, 40 ± 5% relative humidity and photoperiod of 12:12-h (Light/Dark).
All experiments were carried out under the same environmental conditions.

Insecticidal bioassays

Ten adult insects were put into plastic bottles, covered by a fine mesh cloth for ventilation. A cotton wick treated with a mixture of alimentation (sugar solution) and 5, 6, 8, 9, 10, 12 µL of essential oil in 1 mL of acetone was placed in Petri dishes and put into each plastic bottle and covered immediately. Mortality was determined after 72h. Acetone was used as negative control. Each concentration was replicated four times with ten individuals per each replicate in a completely randomized design without subsamples.
Recorded numbers of dead insects among a population treated with biopesticide cloud is not especially the actual number of individuals killed by this biopesticide cloud. For this, we have used Abbott's formula **(Abbott, 1925)** for correcting the mortality rates.
The mortality rates are converted into probits (Ghosh, 1984). These probits are plotted against neperian logarithm to assess the lethal dose (LD_{50}) using the Millerand Tainer method (Miller and Tainter, 1944); DL_{50} and DL_{90} were calculated from the plot of regression lines.

Statistical analysis

All experimental results, represented as mean ± standard error (SE), were subjected to statistical analysis performed by using one-way analysis of variance (ANOVA in XLSTAT software on Microsoft Excel 2007), differences at $p< 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The analytical results and the yields obtained for the different samples are illustrated in **Table 1**. All essential oils have a characteristic odor. The oil extracted by HD has a pale yellow color when the oil obtained by MAHD is colorless.
The percentages yields (based on dry weight (w/w) of essential oils obtained by HD and MAHD are 0.20 % ± 0.015 and 0.30 % ± 0.015 (w/w) respectively.
The reports in the literature revealed a similar yield for the Pakistan grapefruits peel (0.20%) **(Kamal et *al*., 2011).** But significant differences were reported for Nigerian (0.52-0.79%) **(Karioti *et al*., 2012; Okunowo *et al*., 2013)**, Turkish (0.44%) **(Uysal *et al*., 2011)** and Algerian (0.11%) **(Ferhat *et al*., 2016)** grapefruit peel obtained by HD. In addition, the percentage of essential oils obtained by MAHD is higher than that of the Algerian grapefruits peels **(Ferhat *et al*., 2016)** and lowers than that of the Turkish essential oil **(Uysal *et al*., 2011)**.

Table 1 Chemical composition of volatile extracts from *Citrus paradisi* peels obtained by microwave-assisted hydro-distillation (MAHD) and hydro-distillation (HD).

No	Compounds[a]	RT[b]	Class	RI[c]	RI[d]	(%)[e]HD	(%)[e]MAHD
1	α-Pinene	8.73	MH	932	930	0.89±0.01	0.86±0.05
2	β-Pinene	11.03	MH	979	979	1.06±0.03	1.47±0.01
3	β-Myrcene	12.20	MH	998	996	2.99±0.20	3.24±0.20
4	δ-3-Carene	12.97	MH	1010	1009	0.77±0.12	0.90±0.04
5	D-Limonene	15.65	MH	1044	1040	87.51±1.06	85.54±1.50
6	β-Ocimene E	16.27	MH	1058	1051	0.56±0.02	0.86±0.01

7	ϒ-Terpinene	16.8	MH	1064	1059	0.28±0.07	0.06±0.02
8	Cis Linalool oxide	17.82	OM	1081	1075	0.46	0.35±0.03
9	Linalool	19.73	OM	1107	1100	0.41±0,03	0.41±0.04
10	Nonanal	19.89	Others	1112	1109	-	0.09±0.01
11	Citronella	23.33	OM	1159	1153	0.14±0.04	0.14±0.01
12	4-Terpineol	24.81	OM	1181	1179	0.18±0,01	0.16±0.01
13	α-Terpineol	25.86	OM	1196	1195	0.24±0.05	0.23±0.03
14	Decanal	26.78	Others	1213	1206	-	0.50±0.09
15	O-Methylthymol	28.84	Others	1240	1238	0.40±0.02	-
16	Citronellol	28.63	OM	1237	1225	-	0.16±0.01
17	Neral	29.33	OM	1247	1240	0.15±0.01	0.16
18	Geranial	31.34	OM	1277	1272	0.20±0.04	0.23±0.03
19	1-Decanol	31.58	OM	1380	1372	0.16±0.04	0.36±0.04
20	Copaene	37.81	SH	1378	1376	0.16±0.02	0.21
21	β-Cubebene	38.73	SH	1391	1388	0.22±0.08	0.37±0.02
22	Trans Caryophyllene	40.46	SH	1420	1418	0.53±0.02	0.79±0.02
23	Humulene	42.5	SH	1454	1459	-	0.1
24	Farnesene	43.03	SH	1463	1466	-	0.08±0.03
25	Germacrene D	44.19	SH	1482	1485	-	0.19
26	Bicyclogermacrene	45.11	SH	1497	1495	-	0.09±0.02
27	δ-Cadinene	46.78	SH	1527	1529	0.18±0.02	0.30±0.02
28	α-Cadinene	47.2	SH	1534	1537	-	0.08
29	Nootketone	61.91	OS	1812	1800	1.78±0.50	1.80±0.20
Total identified						**99.27**	**99.73**
Yield (%)						**0.20±0.015**	**0.30±0.015**
Monoterpenes Hydrocarbons (%)						**94.06**	**92.93**
Oxygenated Monoterpenes (%)						**1.94**	**2.20**
Sesquiterpenes Hydrocabons(%)						**1.09**	**2.21**
Oxygenated Sesquiterpenes						**1.78**	**1.80**
Others (%)						**0.40**	**0.59**

Note: [a]Compounds identified according their families on HP-5ms column. [b]Retention times. [c]Retention indices given in literature (NIST on non-polar HP5MS™ or DB5 capillary column). [d]Retention indices with respect to C5–C28 n-alkanes calculated on non-polar HP5MS™ capillary column. [e]Percentage calculated by GC–FID on non polar HP5MSTM capillary column. Traces (< 0.05%), Mean ± SD (n = 3)

The volatile compounds identified in both essential oils are listed in **Table 1** according to their elution order on non-polar column as the means of three replicates. Twenty one constituents were identified in essential oils obtained by HD **(Fig.1)** and twenty eight compounds in essential oils obtained MAHD **(Fig.2)**, representing respectively 99.11 % and 99.74 % of the total oil.

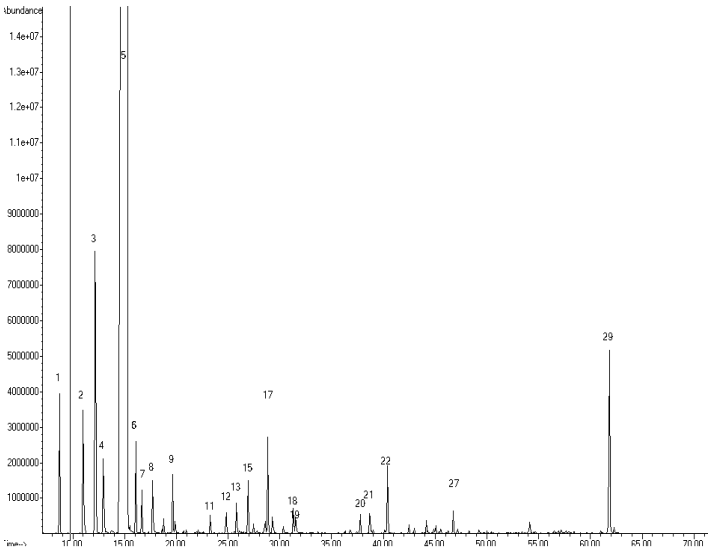

Figure 1 Chromatogram of essential oil of *Citrus paradisi* peels obtained by hydro-distillation (HD).

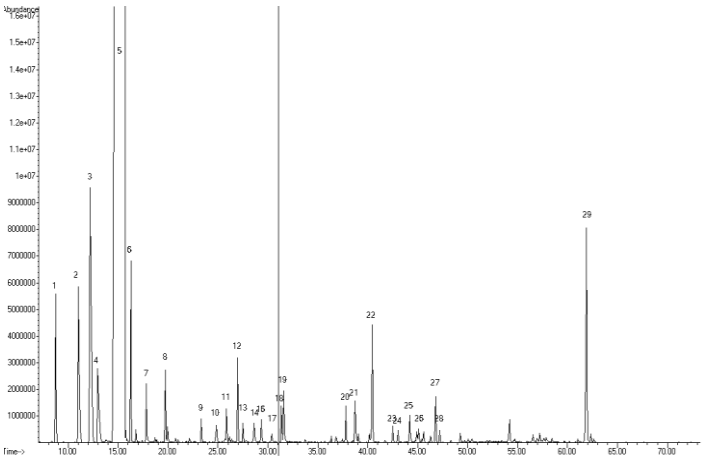

Figure 2 Chromatogram of essential oil of *Citrus paradisi* peels obtained by microwave-assisted hydro-distillation (MAHD).

The monoterpenes hydrocarbons constituted the most dominant chemical group (94.06 % in HD vs 92.08% for MAHD), while the oxygenated monoterpenes (1.7 % in HD vs 1.66 % for MAHD), sesquiterpenes (1.71 % in HD vs 2.21 % for MAHD) and oxygenated sesquiterpens(1.78 % in HD vs 1.8 % for MAHD) showed lower contents.

Limonene was the major compound in HD essential oil (87.51%), followed by β-myrcene (2.99%), nootkatone (1.78%), β-pinene (1.06%), α-pinene (0.89%), δ-3-carene (0.77%), (E) β-ocimene (0.56%), trans-caryophyllene (0,53%). The other important constituents, less than (0.50%) were detected such as linalool (0.41%), cis-linalool oxide (0.46%), neral (0.4%), α-terpineol (0.24%) and β-cubebene (0.22%).

The results for essential oil extracted by MAHD were in agreement with those obtained of HD essential oils. Indeed, the major compound was limonene (85.54%) followed by β-Mycene (3.24%), nootketone (1.80%), β-pinene (1.47%), δ- 3-carene (0.90%), α-pinene (0.86%), β-ocimene E (0.86%) and trans-caryophyllene (0.79%). Other compounds were detected in appreciable amounts,

such as decanal (0.50%), linalool (0.41%), β-cubebene (0.37%), trans-geranial (0.36%), cis-linalool oxide (0.35%), cis-geranial (0.23%), α-terpineol (0.23%), copaene (0.21%), 4-Terpineol (0.16%) and citronella (0.14%).

The various studies on essential oils of grapefruit peel extracted by hydrodistillation showed a wide variety of chemical composition. The major compounds of essential oils of *citrus paradisi* originating from Soudan **(El Kamali *et al*., 2015)**were limonene (74.45%), sabinene (1.21%), α-pinene (3.74%), decanal (1.18%),caryophylene (1.15%) and α-farnesene (1.13%). However, the chemical composition of Italian essential oils **(Caccioni *et al*., 1998)** was dominated by limonene (93.70%) and myrcene (1.86%). The essential oil of Nigeria showed two chemotypes: the first one **(Kariotie*tal*., 2012)**was characterized by limonene (81.86%), α-pinene (2.11%), caryophylene (1.88%), myrcene (7.25%), octanal (1.68%) and β-phellandrene (1.18%), whereas the second one **(Okunowo *et al*., 2013)**was marked by the dominance of limonene (94.2%) and α-pinene (0.7%). The mains constituents detected in Turkey (Uysal et *al*., 2011**)** peels essential oils were limonene (88.6%), β-pinene (1.2%), α-terpinene (1.0%), α-pinene (0.7%), myrcene (0.9%), linalool (0.7%). This composition is relatively comparable to that of our study, indeed the mains componentswere limonene (87.51%), α-pinene (0.89%), myrcene (2.99%), linalool (0.41%) and nootkatone (1,78%). It is interesting to note that nootkatone compound has not been detected in any essential oil previously mentioned in this text. According to investigation of the essential oil of Algerian origin **(Ferhat *et al*., 2016)**, the essential oil of *Citrus paradisi* (variety bouquet of nice) studied was rich in limonene (93.01%), myrcene (1.84%) and linalool (1.40%). It should be noted that the investigation of the essential oils of Pakistan origin **(El Kamali *et al*., 2015)** revealed the presence of nootkatone in appreciable amounts (4.37%-10.9%) according to the mode of drying pretreatment of peels.

In the case of chemical composition of essential oils extracted by microwave, there are only few studies on *citrus paradisi*. The investigation on essential oils of Turkey **(Uysal *et al*., 2011)** revealed the limonene (91.5%) and linalool (1.1%) as mains components. These two compounds were also detected in the Algerian oil **(Ferhat *et al*., 2016)** with similarly percentages, however two other compounds were identified, namely linalyl acetate (1.10%) and myrcene (1.73%). This composition is very different from our one. Indeed limonene (87.51%) and myrcene (2.99%) are the common compounds but we have also detected β-pinene (1.06%) and nootkatone (1.78%) with appreciable amount.

Antimicrobial activity

The antimicrobial properties of essential oils are due to the presence of active constituents, and can be attributed to the monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols **(Griffin *et al*., 1999)**.

Thus, we have undertaken a study on antimicrobial activity of essential oils from *Citrus paradisi* obtained by HD and MAHD. The antimicrobial activity was evaluated by the disc diffusion method against three bacterial strains and four molds (**Table 2**).

The results showed that the grapefruit peel essential oils inhibits the growth of all tested bacteria except for *P.aeruginosa*, which is inhibited only by the essential oil extracted by the conventional method HD. However, these oils inhibited only the growth of *Candida albicans* andthis inhibition depends on both the oil volume used and the nature of strain.

Table 2 Antimicrobial activity of grapefruit essential oils obtained by MAHD and HD methods against bacteria and molds

Essential oils	**MAHD**			**HD**			Erythromycin
Volume (µL)	10	30	50	10	30	50	(30µL /disc)
E. coli	4± 0.50	12 ±1	13±1.22	—	12	15 ±1	33±1 .03
P. aeruginosa	—	—	—	—	12 ±0.50	15 ±1	39±0.50
S.aureus(methicillin resistant)	9	11	13 ±1	10±1	13	15	36±0.75
C. albicans	8 ± 0.60	11 ±1	12	—	17 ±0.90	20 ±1.30	—
V. dahliaeKleb	—	—	—	—	—	—	—
A. niger	—	—	—	—	—	—	—
*Thielaviopsis*spp.	—	—	—	—	—	—	—

— :No inhibition zone was observed. Values are given as mean ± SD of triplicate experiments, Diameter of inhibition zones are expressed in mm

Both essential oils showed the same activity against all bacterial strains with moderate inhibition zones. As for *C. albicans*, the most important inhibition diameter was observed with the oil obtained by HD method (20 mm vs 12 mm). Furthermore, the essential oils exhibited moderate antibacterial activity compared with the control antibiotic, except for *C. albicans* who was not inhibited by Erythromycin.

Several studies have shown the no activity of different essential oils against *P. aeruginosa* **(Cosentino *et al*., 1999; Tepe *et al*., 2005; Matasyoh *et al*., 2007)**.It is important to note that the essential oil of *Citrus paradise* from Turkey showed no effect against this bacterium **(Uysal*etal*., 2011),** which was certainly due to the difference between the chemical compositions of the two oils.

According to the literature, limonene is mainly responsible for antimicrobial activity of the *Citrus* genus oils. So, the antimicrobial activity of the essential oils of grapefruit peel can be attributed to limonene, the main constituent in the two oils **(Uysal *et al*., 2011; Stashenko *et al*., 1996; Merle *et al*., 2004; Sharma *et al*., 2004)**.

Essential oil obtained by HD method was more effective than that obtained by MAHD and this result can be explained by the higher percentage of limonene obtained by HD. Thus, we may conclude that the activity of the essential oil extracted by HD against *P. aeruginosa* is attributed to the synergistic effect between the different components of this oil.

To the best of our knowledge, the results presented here can be considered as the first information on the comparison of antimicrobial activity of the grapefruit peel essential oils from Algeria obtained by MAHD and HD methods.

Insecticidal activity

The major components of plant essential oils, having a low risk for environment and humans, can be used as natural alternatives for conventional insecticide **(Isman, 2000).**

Thus, we have undertaken a study on insecticidal activity of essential oil of *Citrus paradise* peel obtained by hydrodistillation.

In the literature, a toxicity of some essential oil against *C. capitata* (larvae and adults) was examined and showed activity against this specie.**(Giatropoulos *et al*., 2012; Benelli et *al*., 2012)**.

The result showed that the grapefruit peel essential oil has a toxic effect against adults of *C. capitata* with more than 60% of mortality at higher concentrations (10, 12µl/ml). Also at lower concentrations (5, 6 µl/ml), the toxicity of the essential oil was not significant (**Fig.3**), with LD_{50} and LD_{90} values of 9.12 ± 2.5 µl EO/ml of acetone and 13.18 ± 2 µl EO/ml of acetone respectively, (90% of mortality after 72h). Previous studies on the toxicity of three Citrus genus from Greece showed that these essential oils have a toxic effect on larvae of *C.*capitata, doses lower than 0.8 µl/g of food were not active against larvae, while doses higher than 13µl/g of food killed the higher proportion of larvae **(Benelli *et al*., 2012)**.

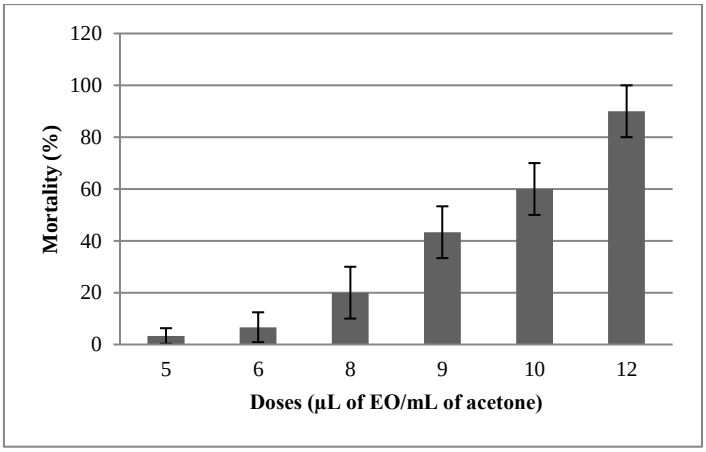

Figure 3 Mortality of adults of *C. capitita* after 72h of treatment with different concentrations of *Citrus paradisi* essential oil, Means (n= 5) using 10 adults per replicate.

In the present research, the insecticidal activity of the essential oil of grapefruit peel can be attributed to limonene, the main constituent of the grapefruit peel essential oil, due to its pronounced toxic effect, as already discussed (**Giatropoulos *et al*., 2012; Papachristos *et al*., 2009; Chantraine *et al*., 1998; Kassir *et al*., 1989; Michaelakis *et al*., 2008)**. Previous research showed that the hydrocarbons limonene, ϒ-terpinene and myrcene are mainly responsible for the toxicity of Citrus genus oil against *C. capitata* with LC50, calculated respectively (6.2, 7, 9.6 µl/g), whereas the pinenes are the least active components **(Benelli *et al*., 2012)**. Therefore, the dominate family of detected volatile were monoterpenes hydrocarbons (94.06%), while the oxygenated monoterpenes (1.7%), sesquiterpenes (1.71%) and oxygenated sesquiterpenes (1.78%) contents

were very low. So, we can conclude that the active components of monoterpenes hydrocarbons can be responsible for the toxicity of grapefruit peel essential oil against the adults*C. capitata*.

CONCLUSION

The composition of the grapefruit peel essential oils obtained by both methods was found to be similar. Both essential oils showed the same antimicrobial activity against all bacterial strains and moulds except for *Pseudomonas aeruginosa*,which was inhibited only by the oil extracted by the conventional method HD. Essential oil of *Citrus paradisi* peel obtained by HD showed an interesting insecticidal activity against *C.capitata*.

Finally, our studies showed important data about the chemical composition, antimicrobial and insecticidal activities of the grapefruit peels essential oil from Algeria, which could be a potential natural flavor additive substituting for chemicals in food preservation and can become an interesting alternative for the conventional insecticide for plant protection.

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